FAT METABOLISM IN THE FISH. II INTESTINAL ABSORPTION AND DISTRIBUTION STUDY OF OIL IN THE CARP, CYPRINUS CARPIO LINNE

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INTESTINAL ABSORPTION AND DISTRIBUTION STUDY OF OIL IN THE CARP, CYPRINUS CARPIO LINNÉ

By

Mitsu KAYAMA and Yasuhiko TSUCHIYA

Department of Fisheries, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received May 12, 1959*)

Introduction

Many kinds of labelled fatty acids have been used in the works of absorption, transportation and distribution studies including acids containing iodine (1), elaidic acid (2), acids containing conjugated double bonds (3), and acids containing isotope, e.g. deuterium (4) or radioactive carbon ^{14}C (5).

Although all these works on fat metabolism have involved the studies of land animals, such as the rat, cat, dog, hen and cow etc., there is no study in the field of fish except for our previous research with the conjugated fatty acids (6). Even if the rats are used as experimental animals for the study of fat metabolism, we could not analogize the results in rats for fish itself. Moreover, since it seems well that there are differences of metabolism among animals, especially in the poikilotherm fish of aquatic animals, such study in the fish becomes necessary. The recent research of Reiser et al. (7) on the origin and metabolism of marine fatty acids must be given attention.

The present study deals with the absorption, transportation and distribution of lipids in the carp by the use of stearic acid-1-^{14}C. The results will be considered comparing with our previous works with the conjugated fatty acids in the carp (6) and the study in the rat of Bergström, Borgström and Rottenberg (8).

Experimental

The stearic acid (100 μc/51.7 mg) labelled in the carboxyl group with ^{14}C has been used as a tracer in this experiment.

Intubation and urethane anesthesia. The present intraintestinal intubation technique of test oil to the carp also followed the device (6) using polyethylene catheter as described in the previous report. The carp being intubated is anesthetized for one to three minutes in 1 per cent urethane solution before

the intraintestinal administration. The urethane anesthesia is more successful than the previous wrapping method in the treatment of intubation to the pre-intestine. The anesthesia is sufficient as soon as the carp stops movement and turns upside-down in the urethane solution. The stearic acid dissolved in cotton seed oil, 0.3 ml (0.2771 g, 22,000 cpm) are poured into the pre-intestine through a polyethylene tube with a syringe. The carp returned to a glass aquarium after the administration treatment attains the normal condition within two minutes.

Sampling and extraction. The one year carp which had hatched out in the last May were used as the test animals. About 100 g of carp caught from a breeding-pond were kept in an aquarium one by one without feeding. The water temperature was 22 to 25°C in the summer season. At each interval after administrating the test oil four carps were sacrificed. First the blood was collected from the caudal part, and the viscera was removed by cutting the belly and separated into the spleen, gall-bladder, gonad, kidney, hepato-pancreas, mesenterial fat, pre-intestine, meso- and post-intestine, and muscle with skin from which the scale was removed.

The tissues and organs were ground with sufficient anhydrous sodium sulphate and the lipids were extracted with ether in small quantity to make the ether extraction thick. The intestine was removed in one piece and freed from the mesenterial fat as far as possible and cut into two parts at the junction of the pre-intestine and meso-intestine. The contents of the pre-intestine and meso-, post-intestines were squeezed out with tweezers as far as possible. The amounts of the active lipids recovered here were used for calculating the total counts of unabsorbed fat adding the lipids present in faeces. The intestine and muscle were subdivided with scissors before grinding.

Direct mount technique. The ^{14}C activity of a fatty acid sample can be readily determined by the direct mount procedure of Entenman, Lerner, Chaikoff, and Dauben (9). This technique avoids the dilution of activity and laboriousness associated with the preparation of barium carbonate mounts. The aluminum disc, 2.5 cm in diameter (originally 1.75 inches disc was used), lined with a piece of lens paper of the same diameter, was weighed and then placed beside a fan. An aliquot of the ether solution containing the ^{14}C-labelled stearic acid was added drop by drop to the lens paper at a rate that kept the surface constantly and uniformly wet but prevented the fat solution from creeping beyond the edge of the disc. The disc and its contents were then reweighed after evaporating the solvent ether and after being dried. The activity of the mounted material was measured by a thin mica window Geiger tube.

Results

From the empirical curve (A) of Entenman et al. we plotted the curve (B) for the conversion factors from the fat directly mounted to the barium carbonate basis as seen in Fig. 1. The plots of these values as ordinates against mg weight of oil used in the direct mount as abscissa are illustrated.

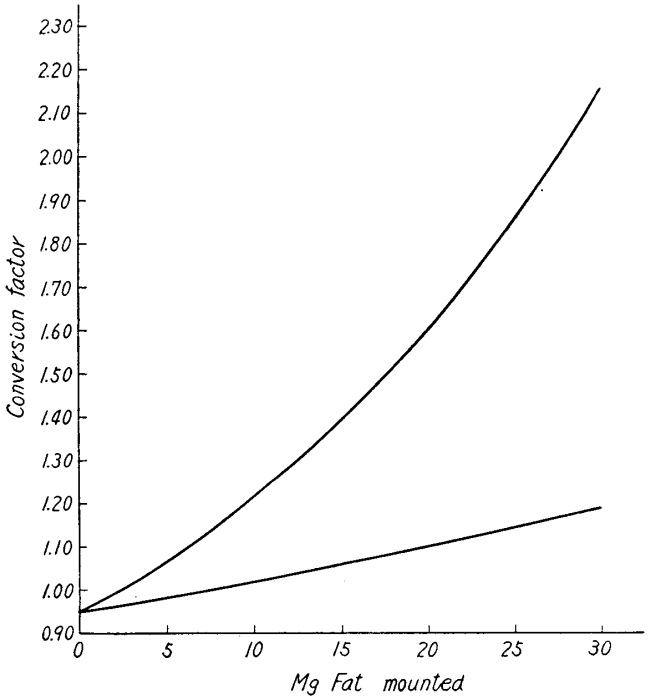

Fig. 1. Factors for conversion of activity to $BaCO_3$ basis by direct mount technique.

Table 1. Examination of counts by direct mount technique.

Different isotope concentration \ Different oil weight		1	2	3	4	5	6	Average
1	Mg oil mounted cpm/mg	1.9 39.2	5.1 39.4	7.2 38.2	7.7 36.6	13.1 39.5	23.6 39.3	38.7±1.13
2	Mg oil mounted cpm/mg	0.8 78.0	0.9 77.7	2.3 78.7	2.3 77.3	3.4 79.9	5.2 79.9	78.6±1.12
3	Mg oil mounted cpm/mg	0.7 111.6	2.1 114.5	2.7 115.3				113.8±2.75
4	Mg oil mounted cpm/mg	2.2 126.6	3.5 126.0	4.1 122.6				125.1±2.16
5	Mg oil mounted cpm/mg	2.6 196.5	3.0 192.7	4.3 193.0				194.1±2.11
6	Mg oil mounted cpm/mg	1.4 223.7	1.5 224.1	4.0 231.2				226.3±4.12

Counts from the directly mounted stearic acid-1-^{14}C were examined for many ether solutions of the different concentrations of labelled stearic acid dissolved in cotton seed oil. Table 1 shows the results of examination of counts by the direct mount technique. The upper value and lower datum in each line respectively represent the oil weight directly mounted in mg and cpm per mg oil. The results correspond well at the different oil weights in the same specific activity.

1. Rate of intestinal absorption.

The rate of absorption of the labelled fat was studied after administration of 0.3 ml (22,000 cpm) per fish. The contents of the pre-intestine and meso-, post-intestines were squeezed out with tweezers. The intestinal contents and the faeces were counted respectively. The intestinal absorption rate is indicated as percentage subtracting the ratio of the total counts present in the intestinal contents and faeces to the total counts fed from 100 per cent.

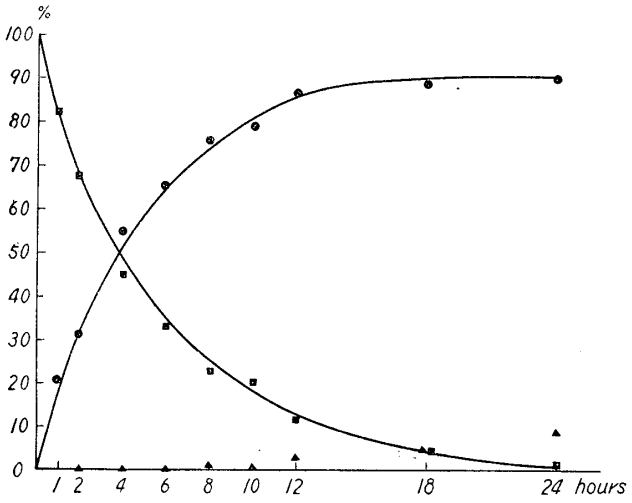

Fig. 2. Intestinal absorption of the labelled oil (●). Per cent of isotope fed present in intestine (■), and faeces (▲).

The intestinal absorption increases reciprocally against the emptying rate of the intestine with the lapse of time as indicated in Fig. 2. About 30 per cent was absorbed in two hours, 50 per cent in four hours, 60 to 70 per cent in six hours and the maximum absorption of 80 to 90 per cent was attained in ten to 12 hours. The curve would be presented as hyperbola. About 10 per cent of the isotope administered appeared in the faeces. It could be pointed out that the intestinal absorption of the carp was rather inferior at 22 to 25°C water temperature comparing with the study of Bergström and his co-workers with the rat (8).

2. Distribution of the absorbed isotope in the lipids of different organs.

The relative specific activities of the lipids present in different tissues and organs were plotted with the lapse of time in Fig. 3.

In the intestines the relative specific activities showed rapid increases up to the maxima after about seven hours in the pre-intestine and eight hours in the meso- and post-intestines when the activities were about 65 per cent

of the acid fed. The curves are almost identical in both parts of the intestines, but the increase occured faster and decreased at a slower rate in the former. This proves that the pre-intestine of the carp, which represents the stomachless fish, acts functionally as the intestine even though it appears like a stomach.

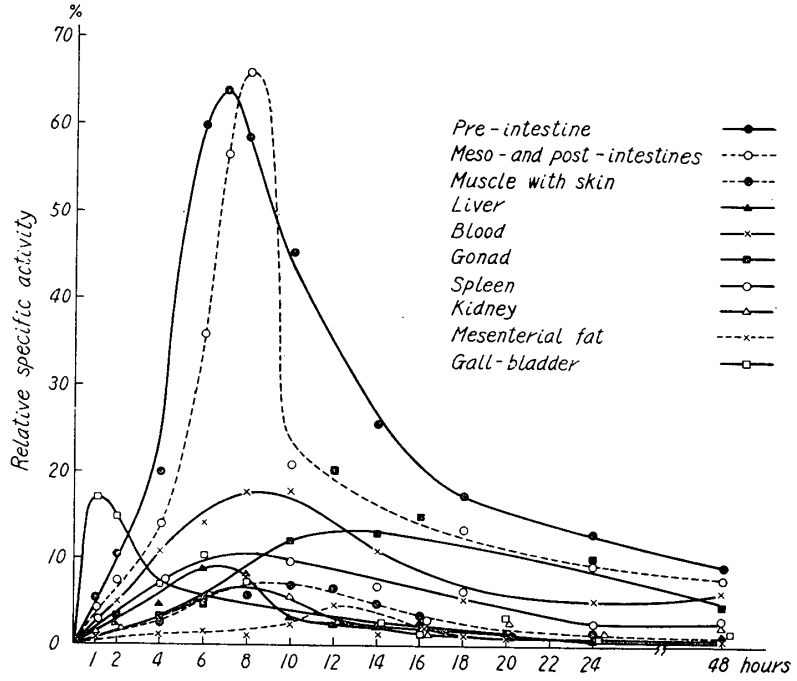

Fig. 3. Relative specific activities of lipids in per cent of oil fed.

The relative specific activity of the blood reached a maximum at the same or somewhat later time as in the intestines. And the maximum relative specific activity is, however, only below 18 per cent. In the liver the relative specific activity after feeding increased as fast as the intestine, however, the maximum was less than 10 per cent. Some of the reasons are discussed in a later section.

As expected the activities in the muscle with skin lipids were low, but showed a slow progressive increase. The fast increase at the initial stage and subsequent rapid decrease were observed in the lipids of the gall-bladder. It seems that there is some flowing backward to the gall-bladder when the labelled oil is intubated into the pre-intestine with a syringe. The relative specific activities in the lipids of the gonad showed a progressive increase beyond expectation.

To determine the distribution of absorbed oil the following calculation was made. The results of distribution at ten hours after administration are shown in Table 2.

In the first and second columns the moisture and crude oil contents are

shown for reference. The distribution of oil present in various tissues and organs is examined as presented in the third column in the average of five carps analyses. The oils in each part of 100 g carp are indicated in the fourth

Table 2. Distribution of absorbed oil at ten hours after administration.

	% Moisture	% Crude oil	Distribution of oil	Oil in 100g carp	cpm/mg / cpm/mg fed	Distribution of absorbed oil at 10hrs.	Remark
			%	g	%	%	
Muscle with skin	80.41	3.18	47.05	1.8538	7.0	46.84	Removed scales
Pre-intestine	80.84	6.39	1.08	0.0425	45.3	6.97	
Meso-, Post-intestines	80.17	3.11	0.62	0.0244	20.6	1.80	
Liver	77.04	3.50	2.17	0.0855	3.2	0.97	
Gall-bladder	83.01	1.11	0.20	0.0079	8.2	0.22	
Heart	84.07	2.69	0.20	0.0079	2.1	0.07	
Kidney	80.66	3.01	0.37	0.0146	3.8	0.22	
Spleen	78.72	5.00	0.87	0.0343	9.7	1.19	
Mesenterial fat	60.31	27.78	3.50	0.1379	2.5	1.26	
Gonad	64.85	8.47	1.40	0.0552	12.4	2.49	Counted as 1.5% of body weight
Blood	85.65	0.56	0.23	0.0091	17.9	0.58	
Intestinal contents	84.27	3.42	1.70	0.0670	87.5	21.15	
Others			40.61	1.6000		16.24	

column. While the relative specific activities of tissues and organs at ten hours are gained from the data of Fig. 3 as seen in the fifth column. Therefore the distribution of absorbed oil at ten hours is calculated dividing the product of the fourth and fifth columns by 0.2771×100 which is the weight of 0.3 ml oil fed times the relative specific activity of labelled oil as presented in the last column of Table 2 as per cent of the administered oil.

Discussion

Bergström and his co-workers have studied the intestinal absorption and distribution of fat in the rat. The results obtained here in the fish will be discussed with the previous investigations in the rat.

The rate of intestinal absorption during the first increasing was rather slower and the appearance of labelled stearic acid in the faeces was also late. This slow rate was observed also in the emptying rate of the intestine. It is an interesting point that these causes are whether solely due to the difference of body temperature between the homoiotherm rat and the poikilotherm fish or due to the functional difference in the intestine. In this experiment the water temperature was 22 to 25°C of the summer season.

Following the intestinal absorption of the labelled fats practically all of

the absorbed oil would be transported via the thoracic duct, not via the portal vein in the higher fatty acids, whether fed as glyceride or as free fatty acids. The comparatively low relative specific activity and distribution in the blood lipids during the absorption of stearic acid-1-^{14}C require some comments. Here we agree with the thinking of Bergström et al. in the rat. The low relative specific activity of blood showed that the labelled acid that enters the blood stream from the lymph was very rapidly absorbed by the various tissues and organs and has not been turnovered with the corresponding lipids (lipoprotein) present in the blood. However, since the conclusions should be made with great caution, the transport of lipids in the systemic circulation will be discussed examining the measurements of glyceride, free fatty acids and phospholipids separately.

As to the low response in the liver it may be considered that the carp contains larger amounts of fats in the hypoderm whereas the cod and shark in the liver and therefore the absorbed fats will be deposited rather in the hypodermic adipose tissues than the liver in the carp.

The fats are absorbed not only in the meso- and post-intestine, but also in the pre-intestine, even though the latter appears like a stomach in the carp with no stomach.

Moreover, a new technique must be deviced to collect the lymph with something like fistula to determine the so-called partition theory of Frazer in the fish.

Summary

The intestinal absorption, transportation and distribution of fat in the carp, *Cyprinus carpio* LINNÉ, have been studied with the aid of stearic acid-1-^{14}C dissolved in cotton seed oil.

The previously reported catheter method has been adopted for the administration of labelled oil to the carp. The urethane anesthesia for the carp was successful in the treatment of intubation to the pre-intestine of the carp. The ^{14}C activities of the lipids present in the various tissues and organs were determined by the direct mount technique.

The intestinal absorption increases reciprocally against the emptying rate of the intestine with the lapse of time. It can be pointed out that the absorption rate of the poikilotherm carp has been slower at 22 to 25°C water temperature of the summer season than of the rat. About 30 per cent were absorbed at two hours, 50 per cent at four hours, 60 to 70 per cent at six hours and maximum absorption of 80 to 90 per cent was attained at ten to 12 hours and about 10 per cent appeared in the faeces.

The relative specific activities of the lipids present in different tissues and organs were hourly plotted in the course of time. The pre-intestine of

the carp which looks like stomach acts functionally as the intestine and absorbs well the administered oil.

To determine the distribution of absorbed fat the calculation at ten hours after feeding has been made.

From this experiment also the so-called IKE KOMI or IKE SHIME custom of Japanese fish culturist has been justified as previously reported.

This research fund was partly defrayed by a grant from the Ministry of Education.

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